Dietary Protein Level-Dependent Alterations in Urinary Excretion of Thiol Compounds Caused by L-Methionine Supplement in Mice

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To determine whether alterations in the L-methionine metabolism depend on nutritional conditions such as dietary protein levels, the effects of an L-methionine supplement for a protein-deficient diet on the urinary excretion of low molecular weight thiol compounds were compared to those of the supplement for an adequate protein diet. Although urinary concentrations of L-cysteine and L-homocysteine were increased by the L-methionine supplement irrespective of the dietary protein levels, the levels of increases were higher by the supplement to the protein-deficient diet than to the adequate protein diet. In addition, the urinary concentration of glutathione was increased only by the L-methionine supplement to the protein-deficient diet. However, the L-cysteinylglycine (CysGly) concentration was not affected by the L-methionine supplement to either diet. Thus, increases by the L-methionine supplement in the concentrations of these thiol compounds, except for CysGly, were more remarkable when the dietary protein level was deficient. These results suggest that the metabolism of excess L-methionine could be markedly affected by nutritional conditions, and that the alterations in the metabolism, at least partly, depend on the dietary protein levels.

Key words ——— dietary protein, L-methionine, L-cysteine, L-homocysteine, glutathione, urinary excretion

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INTRODUCTION

Glutathione (GSH), a ubiquitous thiol-containing tripeptide, plays many roles in the cellular defense of mammalian tissues.[1)] GSH metabolism has been found to be modulated by the levels of sulfur amino acids in culture medium *in vitro*[2,3)] and by dietary levels of protein and sulfur amino acids *in vivo*,[4–7)] since L-cysteine is the late-limiting precursor in GSH biosynthesis.[8,9)] Previous studies using mice have demonstrated that dietary levels of protein and sulfur amino acids markedly affected not only GSH concentrations in several tissues[7)] but also the urinary excretion of thiol compounds.[10)] For example, although L-homocysteine, an intermediate in the metabolism from L-methionine to L-cysteine, was found to be a minor compound compared to L-cysteine and L-cysteinylglycine (CysGly) in urine of mice fed either a 24.8% protein diet (normal protein diet, NPD) or a 7.5% protein diet (low protein diet, LPD), that level drastically increased, reaching a maximum in urine of mice fed an amino acid supplemented diet (ASD) containing 7.5% protein and the same levels of sulfur amino acids as in NPD.[10)] It has been suggested that protein sources and their dietary levels are important factors in an alteration of GSH metabolism caused by a dietary supplement of sulfur amino acids.[4,11)] Accordingly, alterations in the urinary excretion of thiol compounds, including GSH, caused by an L-methionine supplement might be modulated by nutritional conditions such as dietary protein levels.

In the present study, using HPLC after fluorescent labeling, the alterations in the urinary excretion of thiol compounds due to an L-methionine supplement to the diet containing an adequate or deficient level of protein were examined. The data obtained were used to determine whether or not those alterations caused by the L-methionine supplement depend on the dietary protein levels.

MATERIALS AND METHODS

C57BL/6N male mice (age 7 weeks; CLEA Japan Co.; Osaka, Japan) were maintained at 23 ± 2°C and 50–60% relative humidity, and exposed to a 12-hr light cycle from 7:00 a.m. The animals were housed individually and fed on one of four powdered diets [NPD, 1% L-methionine-supplemented normal protein diet (MSNPD), LPD, or 1% L-methionine-supplemented low protein diet (MSLPD)], which

Table 1. Influence of Dietary Protein Levels and L-Methionine Supplement on Urinary Excretion of Thiol Compounds in Mice

Compound	Diet				ANOVA
(μM)	NPD	MSNPD	LPD	MSLPD	
L-Cysteine	$38.36 \pm 4.24^{a)}$	$75.31 \pm 2.76^{b)}$	$25.77 \pm 3.23^{c)}$	$118.52 \pm 40.83^{d)}$	$p < 0.001$
L-Homocysteine	$2.71 \pm 0.29^{a)}$	$27.31 \pm 5.75^{b)}$	$2.41 \pm 0.40^{a)}$	$103.46 \pm 26.46^{c)}$	$p < 0.001$
CysGly	13.55 ± 2.92	16.29 ± 1.63	13.88 ± 1.84	18.71 ± 3.04	NS
GSH	$2.50 \pm 0.54^{a)}$	$2.36 \pm 0.51^{a)}$	$3.42 \pm 0.41^{a)}$	$7.44 \pm 2.26^{b)}$	$p < 0.01$

Abbreviations for diets: NPD, normal protein diet; MSNPD, L-methionine-supplemented normal protein diet; LPD, low protein diet; MSLPD, L-methionine-supplemented low protein diet. The values represent the mean ± S.D. obtained from 3 to 4 mice. NS, not significant. Values with different letters [*a*–*d*)] are significantly different ($p < 0.05$).

composition was reported previously,[6,12] for 5 days before collecting urine. All had free access to each diet and tap water throughout the experiment. All experimental procedures were approved by the Ethics Committee on Animal Experiment of the National Institute for Minamata Disease (NIMD).

The fresh urine collected was deproteinized using 5% perchloric acid containing 1 mM ethylenediamine-N,N,N′,N′-tetraacetate (EDTA). Thiol compounds in each supernatant were fluorescent-labeled using 4-fluoro-7-sulfobenzofurazan, ammonium salt (SBD-F; Dojindo Laboratories; Kumamoto, Japan) according to the method of Toyooka and Imai,[13] and then analyzed by HPLC using a Hibar LiChroCART 250–4 LiChrospher 100 Cica-Merck RP-18(e) (5 μm) column eluted with 75 mM citrate buffer (pH 2.75)-4% methanol with a flow rate of 1 ml/min as reported previously.[10]

Significant differences between individual means were determined by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

It was previously reported that the urinary excretion of low molecular weight (LMW) thiol compounds differed among NPD-, LPD- and ASD-fed mice, especially that of L-cysteine and L-homocysteine,[10] suggesting that these excretions were remarkably affected by a dietary supplement of sulfur amino acids at least to a protein-deficient diet. To clarify whether or not there were dietary protein level-dependent effects of an L-methionine supplement, the urinary excretion of such compounds was determined using mice fed each of the four diets, NPD, MSNPD, LPD and MSLPD. The urinary concentration of L-cysteine was higher in NPD- than in LPD-fed mice, whereas concentrations of the other compounds were similar between the two dietary groups (Table 1) as demonstrated previously.[10] Although an L-methionine supplement resulted in increases in urinary concentrations of L-cysteine and L-homocysteine irrespective of the dietary protein levels, the increases due to the supplement were more remarkable when the dietary protein level was deficient rather than when it was adequate (Table 1). Although the L-cysteine concentration in MSNPD-fed mice was twice as high as that in NPD-fed mice, it was more than 4-fold higher in MSLPD-fed mice than in LPD-fed mice (Table 1). In addition, the L-methionine supplement caused 10- and more than 40-fold higher concentrations of L-homocysteine, respectively, in MSNPD- and MSLPD-fed mice than in NPD- and LPD-fed mice (Table 1). The urinary concentration of GSH was more than 2-fold higher in MSLPD- than in LPD-fed mice, whereas it was similar between MSNPD- and NPD-fed mice (Table 1). In contrast, the concentration of CysGly was not affected by the L-methionine supplement (Table 1). Thus, the levels of increases in the urinary excretion of these thiol compounds, except for CysGly, were higher with an L-methionine supplement to a protein-deficient diet than to an adequate protein diet.

It is well known that most GSH flowing into the renal proximal luminal space through glomerular filtration and the GSH secretion system is decomposed into constituent amino acids including L-cysteine by brush border membrane enzymes such as γ-glutamyltranspeptidase and dipeptidase, and then reabsorbed into the proximal tubule cells.[14,15] Thus, the portion of GSH and its decomposition products such as CysGly and L-cysteine, which escapes decomposition and reabsorption, is excreted into urine.[16] In addition, L-homocysteine, which flows into the luminal space through glomerular filtration, is also reabsorbed into the tubule cells, and only a

little is excreted into urine.[17] In the present study, an L-methionine supplement increased urinary excretion of thiol compounds irrespective of dietary protein levels (Table 1). Given the observations reported in ASD-fed mice,[7,10] the L-methionine supplement would increase in plasma concentrations of GSH and L-cysteine as well as those of L-methionine and its metabolite, L-cystathionine. Since this suggests that the L-methionine supplement would increase the influx of these thiol compounds, such as GSH, L-cysteine and probably L-homocysteine, into the luminal space through glomerular filtration, these increases might overcome the efficient reabsorption, and consequently the urinary thiol compound levels might increase. It should be noted, however, that the L-methionine supplement affected more greatly on the urinary L-cysteine level than the levels of CysGly and GSH (Table 1). Since decomposition capacities are much higher than reabsorption capacities in the renal brush border membranes,[15] this might explain why it is difficult to observe the increases in the urinary levels of CysGly and GSH caused by the L-methionine supplement.

It has been suggested that, due to a lack of essential amino acids, the utilization of sulfur amino acids for protein synthesis is limited, whereas that for GSH synthesis is enhanced.[11] Therefore, similar modulations of L-methionine utilization caused by the deficient dietary protein level might explain the different levels of increases in the urinary excretion of LMW thiol compounds (Table 1). Similar to urinary excretion, an L-methionine supplement might lead to dietary protein level-dependent increases in plasma concentrations of these compounds, since an alteration of methylmercury (MeHg) concentration in the plasma LMW fraction by the L-methionine supplement was dependent on dietary protein levels.[12] In addition, dietary protein level-dependent alterations of L-methionine metabolism caused by the supplement might also be observed in various tissues, as were the cases in plasma and urine as described above.

It has been reported that dietary levels of protein and sulfur amino acids markedly affect the fate of MeHg in mice and rats.[6,10,12,18–20] For example, the urinary excretion of MeHg in ASD-fed mice is much higher than that in NPD- and LPD-fed mice.[10] On the other hand, L-homocysteine, a minor compound in the urine of NPD- and LPD-fed mice, becomes the highest component among four LMW thiol compounds in that of ASD-fed mice.[10] Since urinary excretion of MeHg correlates with that of thiol compounds,[16,21] a marked increase in the urinary excretion of MeHg in ASD-fed mice has been assumed to be explained by an alteration in urinary L-homocysteine excretion.[10] However, urinary excretion of MeHg in MSNPD- and MSLPD-fed mice was identical,[12] although the sum of four thiol compound concentrations as well as the L-homocysteine concentration was higher in the latter than in the former (Table 1). Accordingly, urinary excretion of MeHg would simply not reflect the alterations in urinary thiol compound levels.

In conclusion, the utilization of excess L-methionine might be markedly affected by nutritional conditions such as dietary protein levels, since increases in the urinary excretion of LMW thiol compounds caused by an L-methionine supplement are more remarkable when applied to a protein-deficient diet than to an adequate protein diet.

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